Cytokines and Bone

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List of abbreviations used

OC: osteoclasts

OB: osteoblasts

OS: osteocytes

RANK: receptor activator of nuclear factor κ B ligand

RANK: receptor activator of nuclear factor κ B

OPG: osteoprotegerin

TRAFs; TNFR-associated cytoplasmic factors

M-CSF: macrophage colony stimulating factor

IL: interleukin

TNF: tumor necrosis factor

Th17: T helper 17

gp-130: glycoprotein 130

IFN: interferon

TGF: transforming growth factor

MSC: mesenchymal stem cell

Runx2: Runt-related transcription factor 2

Osx: osterix

Msx2: Msh homeobox 2

BMP: bone morphogenetic protein

Wnt: ingless

Hh: hedgehog

PPAR-γ: peroxisome proliferator-activated receptor gamma

fz: frizzled

LRP5: low-density lipoprotein receptor-related protein 5

GSK-3β: glycogen synthase kinase 3β

SFRPs: secreted frizzled-related proteins

Dkks: Dickkopfs

Sost: sclerostin

Krm: kremen

PKC: protein kinase C

JNK: c-Jun N-terminal kinase

TAK1: TGFβ activated kinase 1

TAB1:TAK1 binding protein 1

Dmp1: dentine matrix protein 1

Pex: phosphate regulating neutral endopeptidase on chromosome X

FGF: fibroblast growth factor

ABSTRACT

Bone turnover is due to cyclic bone resorption followed by bone apposition; these processes are due to the coordinated actions of osteoclasts (OCs) and osteoblasts (OBs). The actions of these two cellular types are orchestrated by osteocytes (OS) that differentiate from osteoblasts and are the most abundant cells in bone. OCs are formed by the attraction of myelomonocytic precursors to the resorption site; the fusion of these cells generates a multinucleated cell attached to the bone surface. OBs derive from a mesenchymal stem cell precursor shared with adipocytes. OS are thought to be the cells primarily responsible for mechanosensing in bone.Numerous cytokines are thought to be responsible for the regulation of bone turnover; most of them have pleiotropic actions and are involved in the regulation of systems other than skeleton. OC formation and function are mainly regulated by the essential factor RANKL, whereas other cytokines increased during inflammation up-regulate OCs and are involved in inflammation-induced bone loss. OB formation and activity are believed to be mainly regulated by the Wnt and BMP signalling pathways.

This review will focus on the main cytokines involved in the regulation of osteoclastogenesis, osteoblastogenesis and coupling of osteoclasts and osteoblasts under physiological and pathological conditions.

INTRODUCTION

Bone is a dynamic tissue that is constantly formed and resorbed in response to changes in mechanical loading, altered serum calcium and pH levels. These changes are mediated by paracrine and endocrine factors. Bone turnover is due to continuous and cyclic bone resorption followed by apposition; these processes are due to the coordinated actions of osteoclasts (OC, which destroy bone) and osteoblasts (OB, which form bone). The actions of these two cellular types may be orchestrated to some extent by osteocytes (OS). In physiological conditions, OB and OC activity is coupled, so that the amount of resorption is equal to the amount of formation. However, in pathological conditions or during senescence, resorption is higher than formation, leading to bone loss. OC and OB formation, as well as the coupling between these two cell types, are mediated mainly through cytokines.

The aim of this review is to describe the complex cytokine network involved in the regulation of bone cells function, and to illustrate the close relationship between the skeleton and other systems, such as the immune system (D'Amelio and Isaia 2009), cardiovascular system (D'Amelio et al. 2009) and adipose tissue (Isaia et al. 2005).

OSTEOCLASTS

OCs are formed by the attraction of myelomonocytic precursors to the resorption site, followed by their fusion, and attachment of the subsequent multinucleated cell to the bone surface (Fig.1 A).

RANKL/RANK/OPG system

A central role in OC biology is played by the receptor activator of NF-kB ligand (RANKL), that is essential for osteoclastogenesis and bone resorption (Leibbrandt and Penninger 2008). Mice and humans deficient in the RANKL gene completely lack OC and exhibit variable forms of osteopetrosis. RANKL has also been implicated in regulation of immune response and in arterial wall calcification (D'Amelio and Isaia 2009, D'Amelioet al. 2009). The functional receptor for RANKL, RANK is encoded by a tumour necrosis factor receptor (TNFR) superfamily gene (TNFGS11A) and is expressed on OC precursors. Mice lacking TNFGS11A have a profound defect in bone resorption and in the development of cartilaginous growth plates. One of the key steps upon activation of the RANK pathway is

the binding of TNFR-associated cytoplasmic factors (TRAFs) to specific domains within the cytoplasmic domain of RANK. The TRAF family proteins are cytoplasmic adapter proteins involved in the mediation of several cytokine-signalling pathways. Different members of the family activate different transcriptional pathways: TRAF2, 5 and 6 are involved in the activation of NF-kB through IkB kinase (IKK) activation and AP-1 through activation of mitogen-activated protein kinases (MAPKs), including Jun-N-terminal kinase (JNK), p38 and extracellular signal-regulated kinase (ERK). Moreover, TRAF6 functions as an ubiquitin ligase, which catalyzes the formation of a polyUb chain. This leads to the activation of IKK and JNK through a proteasome-independent mechanism (Xu et al. 2009) (Fig.1 B-C).

RANKL/RANK signalling promotes the differentiation of OC precursors into mature multinucleated OCs, stimulates their capacity to resorb bone and decreases OC apoptosis. RANKL is present as both a transmembrane molecule and a secreted form, its interaction with RANK is opposed by osteoprotegerin (OPG), a neutralizing soluble decoy receptor, produced by marrow stromal cells and osteoblasts (Grundt et al. 2009). The unbalance between RANKL and OPG has been indicated as the pivotal mechanism responsible for bone loss in case of estrogen deficiency (D'Amelio et al. 2008), inflammation (Leibbrandt and Penninger 2008) and cancer (Fili et al. 2009) induced bone loss.

M-CSF

M-CSF induces the proliferation of OC precursors, their differentiation and increases the survival of mature OCs; OC formation occurs when monocytes are co-stimulated by the essential osteoclastogenic factors RANKL and M-CSF. In addition to RANKL, TNF-α and IL-1 directly regulate NF-kB signalling in OC. As shown in Figure 1 B-C, RANKL activates both classical and alternative pathways of NF-kB; whereas TNF-α and IL-1 activate the classical pathways.

TNF-α

TNF-α enhances OC formation by up-regulating stromal cells production of RANKL and M-CSF, and by augmenting the responsiveness of OCs precursors to RANKL. TNF directly induces marrow precursor differentiation into OCs, although according to some studies it is not osteoclastogenetic in cells not previously primed by RANKL. The ability of TNF to increase the osteoclastogenic activity of RANKL is due to synergistic interactions at the level of NFkB and AP-1 signaling (Fig.1 B). In addition, TNF and RANKL synergistically up

regulates RANK expression. In vivo blockade of TNF in postmenopausal osteoporosis reduces bone resorption (Charatcharoenwitthaya et al. 2007); this suggests that TNF-α increase could be one of the mechanisms responsible for postmenopausal bone loss. TNF is mainly produced by activated T cells and it is also involved in inflammation and cancer induced bone loss both systemically and locally.

IL-1

IL-1 plays an important role in bone loss induced by estrogen deficiency; its level increases after menopause, and is reversed by estrogen replacement. Bone loss does not occur after ovariectomy in mice deficient in receptors for IL-1, and treatment with IL-1 receptor antagonist decreases OC formation and activity. A recent study demonstrates that the blockade of IL-1 reduces bone resorption in postmenopausal osteoporosis (Charatcharoenwitthayaet al. 2007). IL-1 acts by increasing RANKL expression by bone marrow stromal cells and directly targets OC precursors, promoting OC differentiation in the presence of permissive levels of RANKL (Fig.1A). The effect of TNF-α on osteoclastogenesis is upregulated by IL-1.

Additional cytokines are responsible for the up-regulation of OC formation in diseases such as estrogen deficiency, local and systemic inflammation and bone metastasis. Some of these molecules have a well established role in controlling osteoclastogenesis, while others have not. Among these the most involved in OC formation are IL-7, IL-17, IL-23, IL-6, TGFβ and IFNγ. Most of these molecules are also involved in the regulation of immune system and this may explain some of the relationship between immune and bone cells (D'Amelio and Isaia 2009).

IL-7

IL-7 is known for its ability to stimulate T and B cell number and the reaction to antigenic stimuli Recently, a role for IL-7 has also been postulated in bone remodelling (Roato et al. 2006, D'Amelio et al. 2006). We have demonstrated that IL-7 promotes osteoclastogenesis by upregulating T and B cell-derived RANKL (D'Amelio et al 2006) and that the production of IL-7 is downregulated by estrogen.

In humans it has been suggested that IL-7 is osteoclastogenic in psoriatic arthritis and in solid tumors, also in healthy volunteer the expression of IL-7 receptor on T lymphocytes correlates with their ability to induce osteoclastogenesis from human monocytes.

IL-17 and IL-23

IL-17 family members are mainly expressed by a type of human T helper cell (Th17) (Yao et al. 1995). It is now believed that this cytokine plays a crucial role in inflammation and the development of autoimmune diseases such as rheumatoid arthritis,; however, its mechanism of action in the development of bone erosions, especially in relation to other known key cytokines such as IL-1, TNF-α and RANKL remains unclear. Recently, IL-17 has been suggested to be involved in the upregulation of OC formation in inflammation by increasing the release of RANKL, which may synergise with IL-1 and TNF (Lubberts et al. 2005).

One of the stimuli to IL-17 production is IL-23 produced by activated dendritic cells and macrophages. IL-23 drives the T helper 1 response, and is a implicated in autoimmune diseases; hence; it has been suggested that the IL-23/IL-17 axis is critical for controlling inflammatory bone loss. However, in contrast to IL-17-deficient mice, IL-23 knockout mice were completely protected from bone and joint destruction in the collagen-induced arthritis model, indicating that the IL-23-induced bone loss may not be entirely mediated by IL-17, and raising the question whether IL-23 can directly stimulate OCs. Recent work supports this hypothesis suggesting that IL-23 promotes OC formation (Yago et al. 2007). Other recent in vivo studies suggest that IL-23 inhibits OC formation via T cells (Quinn et al. 2008). In physiological conditions (unlike inflammatory conditions), IL-23 favours higher bone mass in long bones by limiting resorption of immature bone forming below the growth plate (Quinnet al. 2008). These contrasting data suggest different roles of this cytokine in the control of physiological or inflammatory bone turnover.

IL6

Activation of the signalling pathway mediated by glycoprotein (gp)-130 by IL-6 and its soluble receptor has been regarded as a pivotal mechanism for the regulation of osteoclastogenesis (Roodman 1992). Nevertheless, in IL-6 knockout mice (IL6KO), as well as in gp-130 deficient mice no decrease in OC formation and function was found. These data may suggest that IL-6 is not essential for bone resorption. However, IL6KO mice were protected against ovariectomy induced bone loss, and this finding, together with the observation of increased level of IL-6 after menopause in women, may suggest a peculiar role for IL-6 in bone loss due to estrogen deprivation. IL-6 was also shown to be involved

in other diseases associated with accelerated bone turnover such as Paget's disease of bone, multiple myeloma, rheumatoid arthritis and renal osteodystrophy.

IFNγ

The effect of IFNγ on OC formation and activity is controversial. IFNγ behaves like an anti-osteoclastegenic cytokine in vitro (Takayanagi et al. 2000), in vivo in nude mice (Sato et al. 1992) and in a knock out models in which the onset of collagen induced arthritis is more rapid, as compared with wild type controls. These data are not confirmed by studies in humans and in experimental models of diseases that indicate an increased level of IFNγ during estrogen deficiency.

In humans IFNγ is positively correlated with bone erosions in leprosy and rheumatoid arthritis. Data from randomized controlled trials have shown that IFNγ does not prevent bone loss in rheumatoid arthritis. The use of IFNγ in humans has been suggested to employ IFNγ for the treatment of osteopetrosis, in which condition IFNγ is able to restore bone resorption.

Taken together, the data in humans suggest that, in some conditions, IFNγ stimulates bone resorption. These discrepancies could be explained by the fact that IFNγ directly blocks OC formation targeting maturing OC and induces antigen presentation and thus T cell activation in vivo. Therefore, when IFNγ levels are increased in vivo, activated T cells secrete pro-osteoclastogenic factors and this activity offsets its anti-osteoclastogenic effect.

TGFβ

TGFβ plays a complex role in osteoclastogenesis. It has wide ranging effects and it has been suggested that it may play a pivotal role in the growing skeleton contributing the coupling between OB and OC (Massague 1990). Three isoforms of TGFβ have been described (TGFβ1–3), which all interact with the same receptor complex. TGFβ1 is mainly expressed in lymphoid organs and in serum. Conversely, TGFβ2 and TGFβ3 are predominantly expressed in mesenchymal tissues and bone. TGFβ is produced by many cell types, including bone marrow cells, OBs and stromal cells and is secreted in a latent form that must be activated to mediate its effects. Although several mechanisms of activation in vivo have been proposed, the precise mechanism of this process is not known. Both in vitro and in vivo studies have shown that TGFβ1–3 have complex effects

on bone. They stimulate or repress proliferation or formation of OBs and Ocs, depending on cell types and culture conditions used. Mice with OB-specific over-expression of TGFβ2 develop high turnover osteoporosis.

TGFβ has also been implicated in the pathogenesis of ovariectomy-induced bone loss because local injection of TGFβ1 and TGFβ2 prevent bone loss at the site of the injection in ovariectomy rats. Furthermore, estrogen is known to upregulate the expression of TGFβ in murine OBs, bone extracts and bone marrow cells and long-term in vivo estrogen treatment has been shown to increase serum TGFβ1 and TGFβ2 levels in humans. Latent TGFβ is abundantly present in the bone matrix and is released and activated during bone resorption, and it feeds back to modulate OB and OC activity. In particular TGFβ is believed to induce OCs apoptosis that follows bone resorption in vivo.

Figure 2 summarizes the action of the above mentioned cytokines on OCs.

OSTEOBLASTS

Osteoblasts are the cells responsible for the production of bone matrix components such as type I collagen. OBs and adipocytes differentiate from a common precursor, the pluripotent mesenchymal stem cell (MSC) found in bone marrow and adipose tissue. Numerous transcription factors and multiple extracellular and intracellular signals regulating adipogenesis and osteoblastogenesis have been identified and analyzed in the recent years. Over the last two decades, many factors have been identified that regulate differentiation. Runt-related transcription factor 2 (Runx2), Osterix (Osx), Msh homeobox 2 (Msx2), bone morphogenetic proteins (BMPs), Wnt and Hedgehog (Hh) have been shown to be critical in osteoblastogenesis ((Marie 2008)) (Fig.3).

Differentiation factors controlling osteoblastogenesis inhibit adipogenesis, and vice versa. In MSC, the Wnt signaling pathways induce osteoblastogenesis, and stimulate OB proliferation and maturation. PPAR-γ a member of the nuclear hormone receptor superfamily, induces MSCs to differentiate into adipocytes (Fig.3). The actions of Wnt and PPAR-γ in osteoblastogenesis and adipogenesis have been extensively studied; however, their molecular interactions and the effects of this interaction have remained unclear.

Wnts

Wnts are a family of secreted lipid-modified proteins that bind to a receptor complex comprising frizzled (fz) and the low-density lipoprotein receptor-related proteins 5 or 6

(LRP5 or LRP6). Activation of this receptor leads to inactivation of glycogen synthase kinase 3β (GSK-3β), which prevents the proteosomal degradation of the transcriptional co-activator β-catenin and, thereby, promotes its accumulation in the cytoplasm. β-catenin translocates into the nucleus and regulates the expression of Wnt target genes (Fig.4). Several molecules serve as antagonists of Wnt signaling: secreted frizzled-related proteins (SFRPs) act as soluble decoy fz receptors by preventing binding of Wnt to fz. Dickkopfs (Dkks) and sclerostin (Sost) bind to and inactivate signaling from LRP5/LRP6 through Kremen (Krm) (Fig.4) (Macsai et al. 2008). Besides this so-called canonical pathway, Wnts can signal via the protein kinase C (PKC), Rho- or c-Jun N-terminal kinase (JNK) The role of this pathway in bone biology is still unclear. Wnt canonical signalling is a critical determinant of bone mass, indeed, loss or gain of function mutations in LRP5 leads to osteoporosis-pseudoglioma syndrome or the hereditary high bone mass trait, respectively.

BMPs

The bone morphogenetic proteins (BMPs) are cytokines belonging to the TGFβ family. More than 20 BMPs have been identified to date and these are divided into classes based on their sequences and functions. Some BMPs (-2, -3, -4,-6, -7, -8 and -8b) are produced by preosteoblasts, while others are secreted by non-parenchymal cells of the liver (BMP-9) or by human endochondral and intramembranous bone. BMP-2, -6, -7 and -9 regulate the proliferation, differentiation and apoptosis of bone cells.

BMPs act on the cells involved in the bone ossification via two pathways: the canonical Smad pathway and a pathway involving the mitogen activated protein kinase (MAPK), (Senta et al. 2009) (Fig. 5). The Smad pathway is activated with the binding of BMPs to their heterotetrameric receptors. Smads form a complex that translocates into the nucleus and binds to a specific DNA sequence and to proteins involved in target gene expression, and activates OB-specific genes. The Smad pathway is regulated by the breakdown of Smad by the ubiquitination of the protein via the Smad-ubiquitination regulatory factor (Smurf1), and the subsequent proteasome proteolytic pathway. It can also be inhibited by extracellular antagonists of BMPs such as noggin and chordin. Intracellular I-Smad can interact with the type I receptor to block R-Smad phosphorylation. Smad 6 also competes with Smad 4, which inhibits the formation of R-Smad/Smad 4 complexes. The Wnt/β-catenin pathway is also involved in the regulation of the Smad cascade by inhibiting GSK3.

The non canonical MAPK pathway involves three different cascades: an extracellular signal-regulated kinase (ERK), JNK and p38 MAPK. Some subgroups of BMPs, can activate the TGFβ activated kinase 1 (TAK1)/TAK1 binding protein 1 (TAB1) complex, and thus activate p38. Activation of this pathway also increases the degradation of Smad.

TGFβ

TGFβ can act on OB, promoting osteoprogenitor proliferation and inhibiting terminal differentiation, by repressing the function of Runx2 (Alliston et al. 2001). TGF*β* also regulates osteoblast expression of osteoclast regulatory factors M-CSF, RANKL and OPG. Currently, TGF is thought to be involved mainly in coupling the actions of OC and OB and to play a fundamental role in the developing skeleton, whereas its role in physiological bone remodelling remains unclear.

PPRγ

PPAR-γ is a member of the nuclear receptor gene superfamily of ligand-activated transcription factors. Two known isoforms of PPAR-γ (1 and 2) are generated by alternative promoter usage and splicing, with PPAR-γ2 being predominant in adipose tissue. PPAR-γ1 is expressed at lower levels in adipose tissue and in other cell types. The expression of both PPAR-γ1 and PPAR-γ2 is elevated during adipogenesis, and both isoforms can induce adipocyte differentiation and are antiosteoblastogenic. In particular, treatment with TNF-α or IL-1 inhibited Troglitazone (Tro)-induced transcriptional activity of PPAR-γ, demonstrating that cytokines may interfere with differentiation of MSC (Suzawa et al. 2003). Canonical Wnt signalling seems to stimulate osteoblastic differentiation without affecting adipo-/osteoprogenitor cells maturing into adipocytes. However recent studies have shown that noncanonical Wnt signalling may directly regulate the transactivation function of PPAR-γ in MSCs (Takada et al. 2007)

Figure 6 summarizes the regulation of transcriptional factors during osteoblastogenesis by multiple signals.

OSTEOCYTES

OSs are derived from Obs. It is not clear why some OBs are designated to become osteocytes, but they make connections with existing embedded cells and then are

engulfed in osteoid. OSs make up more than 90–95% of all bone cells in the adult skeleton, whereas OBs compose less than 5% and OCs less than 1%. Osteocytes are viable for years, even decades, whereas OBs live life-times of weeks and OCs of days. The unique feature of OSs is the formation of long dendritic processes that connect OSs within their lacunae with cells on the bone surface and into the bone marrow. Osteocytes send signals modulating both bone resorption and formation, but how this happens is still unclear. It has been suggested that dying osteocytes send signals of resorption (Verborgt et al. 2002, Kurata et al. 2006) and recently it has been shown that osteocytes can target OB trough Sost and thus inhibit bone formation (van Bezooijen et al. 2005). OSs are thought to drive bone remodelling by sensing mechanical strain along their dendritic processes, the cilia and the cell body.

Osteocytes influence bone remodelling through cytokines as Sost, M-CSF and RANKL. Sost, a Wnt antagonist, is a marker for late embedded osteocytes and its mutation in humans results in sclerostosis (Fig.4). There are also three key molecules expressed in osteocytes that play a role in phosphate homeostasis: dentine matrix protein 1, (Dmp1), phosphate regulating neutral endopeptidase on chromosome X (Pex) and FGF23. Deletion or mutation of either Pex or Dmp1 results in hypophosphatemic rickets with elevation of FGF23. FGF23 is a phosphaturic factor that prevents reabsorption of Pi by the kidney leading to hypophosphatemia (Fig.7). Some data suggest that Dmp1 could function intracellulary to regulate transcription and extracellulary to regulate mineralization of osteoid.

Based on these observations it has proposed that the OS network can function as an endocrine gland to regulate phosphate homeostasis. As both Dmp1 and Pex are regulated by mechanical loading, it will be important to determine if skeletal loading can play a role in mineral and phosphate metabolism ((Bonewald 2007)).

In summary, bone is a living tissue in which three main cells types act in response to multiple signals. The hormones and the cytokines involved in bone turnover have pleiotropic functions that may help explain the complex relationships between skeleton and immune and cardiovascular systems. Future studies will allow us to clarify these relations and to deeply understand signals involved in bone metabolism.

Key points

Simplified diagram of the interactions between bone cells and other organs and systems, these complex pathways are mediated through multiple cytokines.

Key terms

- **Osteoclast:** cells that resorb bone and derived from myeloid precursors.
- **Osteoblast:** cells that form new bone and derived from the MSC.
- **Osteocyte:** the most abundant cells in bone that orchestrate physiological bone remodelling; they are the mature form of osteoblasts.
- **RANKL/RANK/OPG system:** the key system controlling osteoclasts, OPG is the decoy receptor of RANKL and inhibits osteoclastogenesis and activity.
- **Wnt signalling:** together with BMPs is the key system controlling osteoblasts; it acts on OB commitment and activity.
- **BMPs signalling:** together with Wnt is the key system controlling osteoblasts; it acts on OB commitment and activity.
- **Mechanotransduction:** the feature that allows OS to sense mechanical load and to orchestrate bone remodelling and mineral homeostasis according to its variations.

SUMMARY

- Bone is a dynamic tissue and its turnover is due to cyclic bone resorption followed by apposition; these processes are due to the coordinated actions of osteoclasts and osteoblasts, orchestrated by osteocytes.
- Osteoclast and osteoblast action is normally coupled in physiological conditions, whereas in pathological conditions their imbalance leads to bone loss.
- Osteoclasts are formed the fusion of myelomonocytic precursors. Their recruitment, fusion and activation are mainly regulated by the RANK/RANKL/OPG and the NFκB pathway.
- Osteoclast formation is upregulated by estrogen deficiency, inflammation and cancer and hypoxia.
- Osteoblasts differentiate from MSC as well as adipocytes: a central role in osteoblasts biology is played by the transcriptional factors that influence the MSC fate.

- The main cytokines involved in osteoblastogenesis and activity are Wnts and BMPs.
- Osteocytes are the most abundant cells in bone, they are thought to regulate bone remodelling mainly by sensing the mechanical load.
- Several cytokines produced by osteocytes affect bone formation and bone resorption. Recently, it has been proposed that these cells regulate mineral homeostasis by specific cytokines.

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Figure 1. **Central role of NF-kB in osteoclastogenesis, regulation by RANKL, IL-1 and TNF-α.**

A. Key steps of OC formation and activity.

B. RANKL, IL-1 and TNF-α activate classical pathways of NF-kB in osteoclast-like cells.

C. RANKL activates non classical pathways of NF-kB in osteoclast-like cells. Adapted from (Xuet al. 2009)

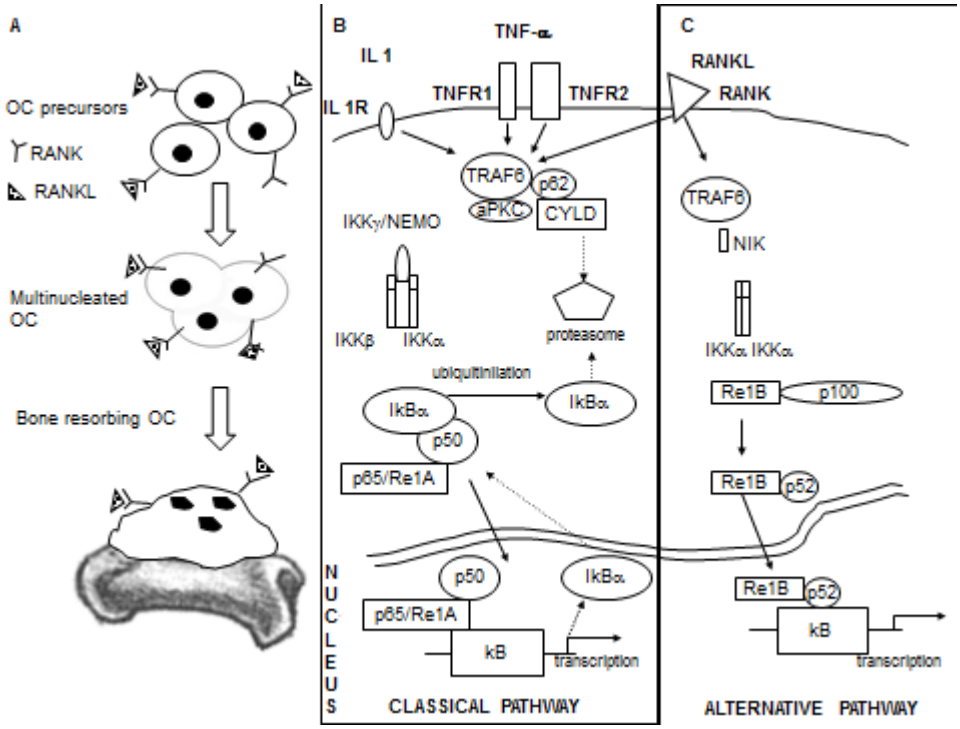

Figure 2. Cytokines involved in OC formation and activity.

Schematic representation of the main cytokines involved in OC formation and activity, the dotted lines represent the more controversial pathways, while the continuous lines represent the pathways with more concordant data.

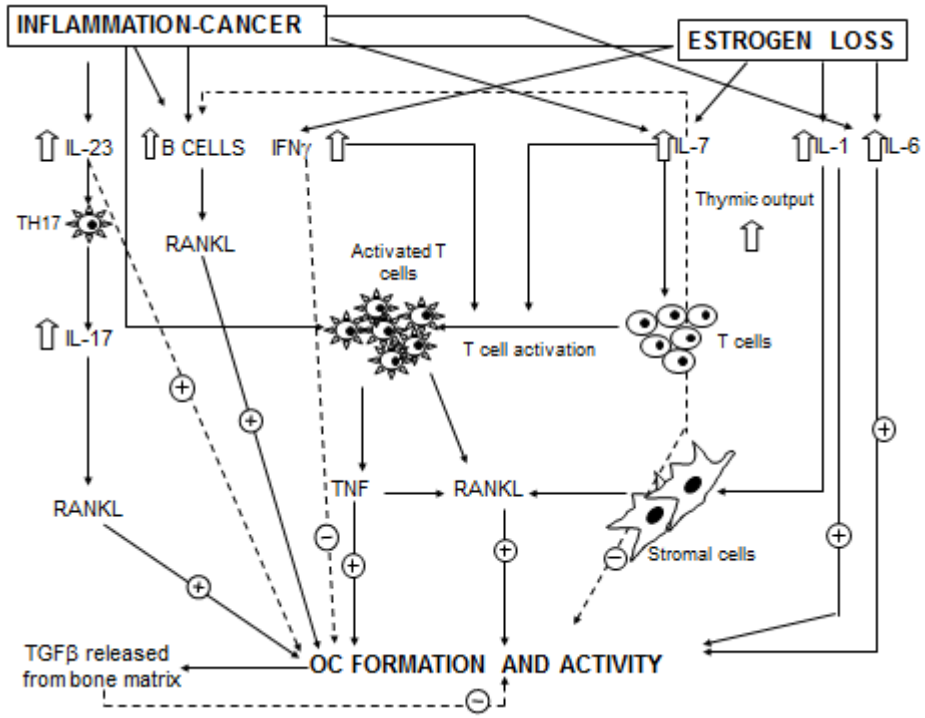

Figure 3. Control of osteoblast differentiation by transcription factors.

OB differentiation from the MSC, with the main transcription factors control involved in each step. Adapted from (Marie 2008).

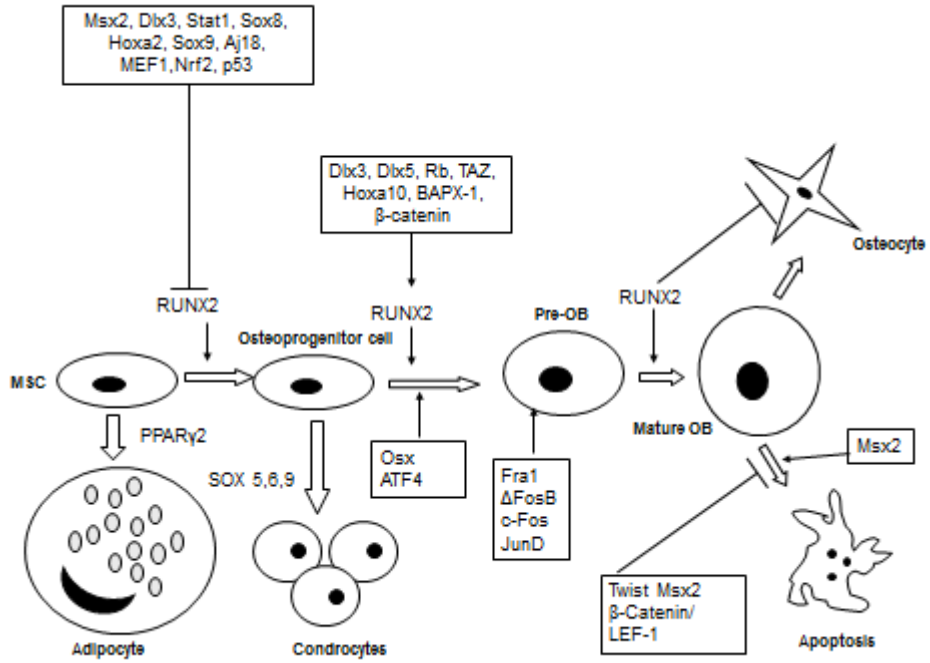

Figure 4. Canonical Wnt pathway and its repressors.

A. Activation of the canonical pathway is initiated when Wnt binds to Fz receptors and low LRP-5/6 co-receptors.

B: Inactivation of canonical pathway through a variety of inhibitors mediates the proteasomal degradation of β-catenin via its phosphorylation. Adapted from (Macsaiet al. 2008).

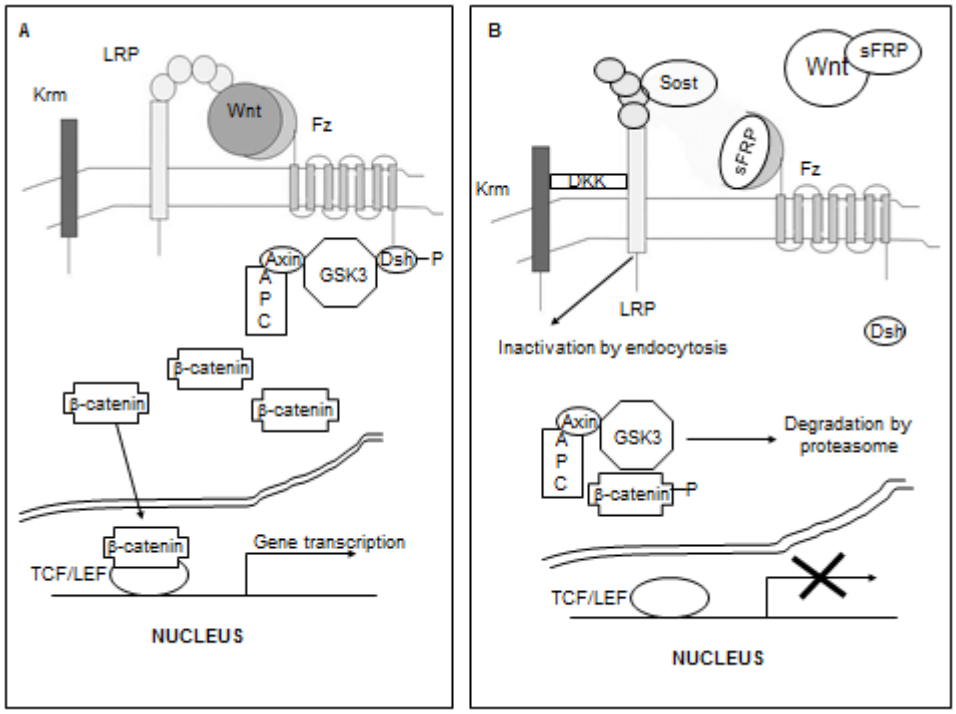

Figure 5. BMPs pathways.

Classical BMP signalling pathwaysthrough Smad and MAPK, adapted from (Sentaet al. 2009).

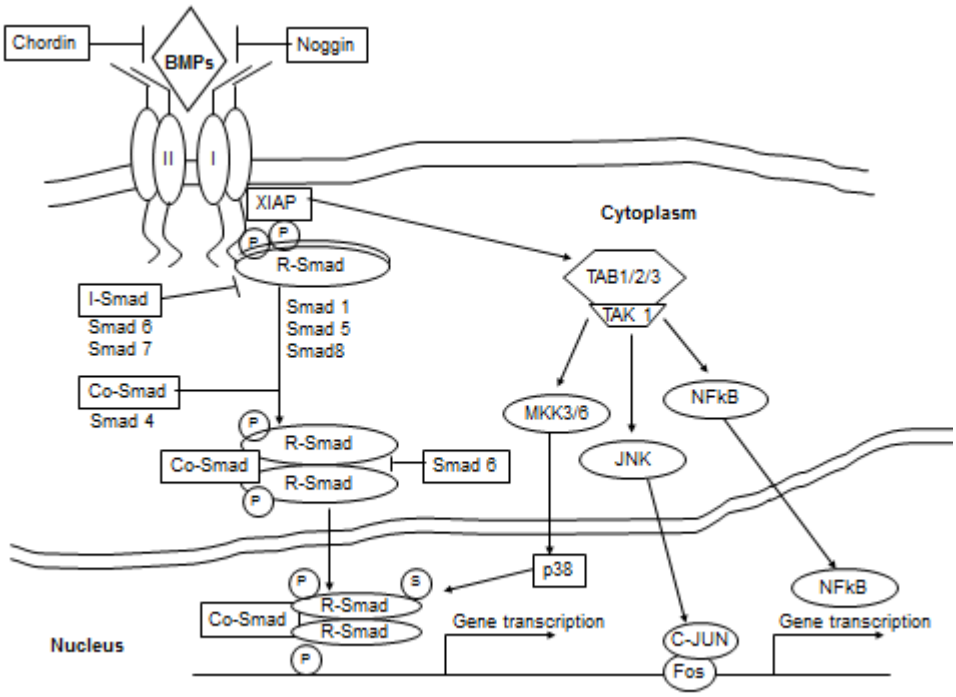

Figure 6. Regulation of transcriptional factors during osteoblastogenesis.

Schematic representation of the signals involved in OB differentiation. Adapted from (Marie 2008).

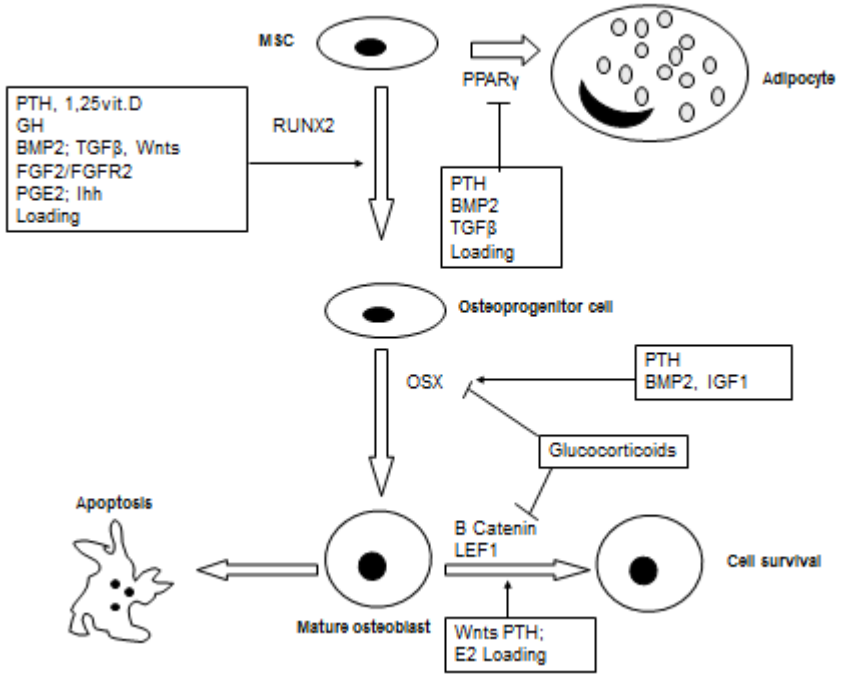

Figure 7. Role of OSs in mineral metabolism.

Interactions between Dmp1, Phex, and FGF23, adapted from (Bonewald 2007).